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Apotrachelocerca arenicola (Kahl, 1933) n. g., comb. n. (Protozoa, Ciliophora, Trachelocercidae): Morphology and Phylogeny

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ABSTRACT. During faunistic study on psammophilic ciliates along the coast of Qingdao, China, a population of *Trachelocerca arenicola* Kahl, 1933 was found and then investigated using silver staining and gene sequencing methods. The results indicated that it represented a new genus *Apotrachelocerca* characterized by uninterrupted circumoral kineties composed of two rows of dikinetids and no brosse or ciliary tuft in the oral cavity. This new genus should be assigned to the family Prototrachelocercidae Foissner, 1996. Based on the small subunit rRNA gene sequence, phylogenetic trees revealed that *Apotrachelocerca arenicola* occupied a basal position to other trachelocercids.

Key Words. *Apotrachelocerca*, Karyorelictea, new genus, phylogeny, Prototrachelocercidae, SSU rRNA gene, taxonomy.

CILIATES belonging to the order Trachelocercida Jankowski (1978), the largest group within the class Karyorelictea, are very common in marine interstitial environments (Alekperov et al. 2007; Al-Rasheid 1996, 1997, 1998, 2001; Al-Rasheid and Foissner 1999; Carey 1992; Dragesco 1960). Although the first report was 200 yr ago (Müller 1786), it was only in the 1980s that detailed descriptions of the infraciliature of this group of ciliates became available (Dragesco and Dragesco-Kernéis 1986; Foissner 1996, 1997, 1998; Foissner and Al-Rasheid 1999a, b; Foissner and Dragesco 1996a, b; Wilbert 1986). With the help of a new ''fixative'' invented by Foissner and Dragesco (1996a) and a protargol staining method modified by Wilbert (1975), more and more new taxa were erected (Foissner 1996, 1997; Foissner and Al-Rasheid 1999a; Foissner and Dragesco 1996a), which might suggest that true diversity of this group of karyorelictids needs further extensive sampling and investigation.

Historically, the generic classification of trachelocercids was very bewildering owing to an accumulation of taxonomic and nomenclatural problems (Dragesco 1960; Kahl 1933). About 15 yr ago, Foissner (1996) and Foissner and Dragesco (1996a) suggested an approach to generic distinction and improved diagnoses for known valid genera. Foissner (1996) argued that the main critical characters used for genus identification should include: brosse (present/absent), ciliary tuft in oral cavity (present/absent), and circumoral kinety (interrupted/uninterrupted; simple/complex). After the establishment of *Sultanophrys*, another character should be included in genus distinction, which is the position of an anterior secant system (at left/right side of glabrous stripe). Based on these characters, six genera have been assigned to the order Trachelocercida. These are *Prototrachelocerca* Foissner (1996),*Tracheloraphis* Dragesco (1960), *Trachelocerca* Ehrenberg, 1840, *Trachelolophos* Foissner and Dragesco (1996a), *Kovalevaia* Foissner (1997), and *Sultanophrys* Foissner and Al-Rasheid (1999a). Molecular information for karyorelicteans became available only recently and about 12 trachelocercid morphotypes have small subunit (SSU) rRNA gene sequence information (Andreoli et al. 2009; Gao et al. 2010b; Mazei et al. 2009), generally confirming these generic distinctions.

Of about 70 trachelocercids described so far, most have been described based on superficial live observations and/or traditional histological methods, which do not reveal the infraciliature. So the identity of these species is still in doubt and detailed redescriptions will be important (Carey 1992; Dragesco 1960; Foissner 1998). Thus, we reinvestigated a population of *Trachelocerca arenicola* Kahl,1933 found near Qingdao, China. In this paper, we establish a new genus for this species and place it in the family Prototrachelocercidae Foissner 1996 based on studying its live morphology, its infraciliature, and its phylogenetic relationships based on SSU rRNA gene sequence.

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MATERIALS AND METHODS

Morphological studies. Materials were sampled from the intertidal zone of the Shilaoren sandy beach at Qingdao (36°10′N, 120°47′E), China on October 9, 2009, when the water temperature was 22 °C and salinity was 32‰. The sampling method was mainly according to Fan et al. (2010). Living cells were studied by bright field and differential interference contrast microscopy (100—1,000X magnifications). The infraciliature was revealed by the protargol impregnation method (Wilbert 1975) using the fixative mentioned by Xu et al. (2011). Counts and measurements of stained specimens were performed at a magnification of 1,000X. Drawings were made with the help of a camera lucida. Terminology is according to Foissner (1996). Systematic arrangement is mainly according to Foissner (1998) and Lynn (2008).

Phylogenetic analyses. DNA extraction, PCR amplification, SSU rRNA gene cloning, and sequencing were performed according to Zhang et al. (2010). The primers 82F (5′-GAA ACT GCG AAT GGC TC-3′) and Euk B (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) were used to amplify the SSU rRNA gene.

Other than the newly sequenced SSU rRNA gene of *Apotrachelocerca arenicola*, the sequences used in the present analyses were obtained from the NCBI GenBank database. The final alignment of 1,721 characters and 56 taxa was used to construct phylogenetic trees according to the methods reported by Gao et al. (2010a). Briefly, the Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using GTR+I+G as the best model selected by the program MrModeltest v.2.0 (Nylander 2004). The ML tree was constructed with the PhyML V2.4.4 program (Guindon and Gascuel 2003) using the best model GTR+I (= 0.3951)+G (= 0.6201) selected by the program Modeltest v.3.4 (Posada and Crandall 1998).

RESULTS

Description of living morphology (Table 1 and Fig. 1, 3–5, 9, 14–24). Cells in vivo are about 300–600 × 10–20 μm, flexible, and contractile (Fig. 1, 3, 4, 14–19); shape is flattened ribbon-like (up to 3:1) including oral area (Fig. 20). Mostly, the body is of almost the same width in anterior three quarters of cell, and no distinct neck; posterior quarter is gradually narrowed and forms a curved tail (Fig. 1, 3, 14–16, 18). Cytoplasm is colorless and transparent except for opaque anterior quarter packed with

Table **1.** Morphometric data from *Apotrachelocerca arenicola* (Kahl 1933) n. g., n. comb.

Character	Min	Max	Mean	SD	CV	*n*
Body length	85	202	120.7	30.9	25.6	26
Body width at head	6	12	8.4	1.6	19.0	26
Body width at trunk	14	38	23.4	5.3	22.7	32
Somatic kineties (number)	7	7	7.0	0	0	20
Macronuclei (number)	1	3	2.3	0.5	23.3	23
Micronucleus (number)	1	1	1.0	0	0	32

All data are based on protargol-impregnated specimens. Measurements in μm.

CV, coefficient of variation in %; Max, maximum; Mean, arithmetic mean; Min, minimum; *n*, number of specimens investigated; SD, standard deviation of the mean.

cytoplasmic granules, which are ellipsoid, about 1–5 μm long, colorless to yellowish (Fig. 9, 14, 16, 18). Cortical granules are round, colorless, and about 0.5 μm in diameter, scattered between ciliary rows and in glabrous stripe (Fig. 5, 23). Cell surface is rather rough (Fig. 20, 21, 24). Contractile vacuole is absent.

Locomotion is by sluggish gliding along bottom of Petri dish.

Infraciliature (Table 1 and Fig. 6–8, 10–13, 25–30). The entire infraciliature consists of dikinetids (Fig. 12, 13). Only right side is ciliated, while left one is occupied by glabrous stripe (Fig. 13, 30). Cilia are about 8 μm long and arranged in longitudinal rows (Fig. 12). The anterior ends of the ciliary rows are not distinctly curved and condensed as in other trachelocercids (Fig. 10, 26). At the margin of the glabrous stripe there is no ciliary row shortened anteriorly or posteriorly. In other words, there is no anterior or posterior secant system formed at the margin of the cell (Fig. 10–13, 25, 30). The glabrous stripe is bordered by a bristle kinety, which is comprised of one row of dikinetids (Fig. 11, 13, 25, 29, 30). Circumoral kineties are composed of two rows of uninterrupted, obliquely, and narrowly spaced dikinetids; the dikinetids of the upper row rotate anticlockwise, while those of lower row rotate clockwise (Fig. 10, 11, 25, 26).

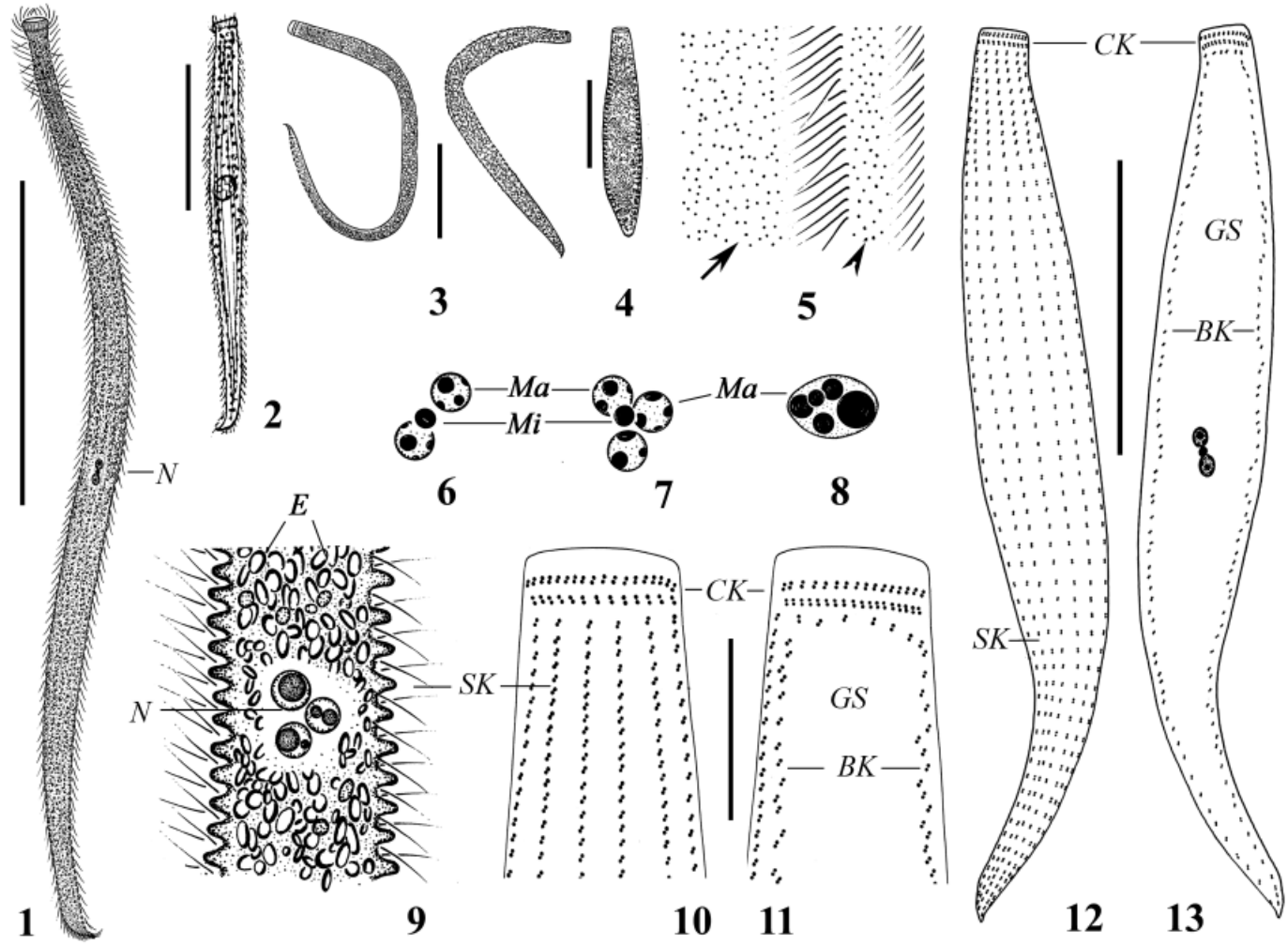

Fig. **1–13.** *Apotrachelocerca arenicola* (Kahl 1933) n. g., comb. n. from life (1–5, 9) and after protargol impregnation (6–8, 10–13). **1.** Right side view of a typical individual, noting nuclear group. **2.** From Kahl (1933). **3.** Flattened and flexible body shapes. **4.** A contracted specimen. **5.** Detail of mid-region of cell to show the distribution of cortical granules between ciliary rows (arrowhead) and in glabrous stripe (arrow). **6–8.** Variation in composition of nuclear group. **9.** Middle region of slightly contracted cell marking ellipsoid (crystalline?) inclusions and nuclear group. **10, 11.** Right (10) and left (11) side view of anterior body region, indicating glabrous stripe, somatic kineties, bristle kinety, and continuous (uninterrupted) circumoral kineties consisting of two rows of dikinetids. **12, 13.** Infraciliature of right (12) and left (13) side, noting left side almost occupied by the glabrous stripe and no anterior secant system. BK, bristle kinety; CK, circumoral kineties; E, ellipsoid inclusions; GS, glabrous stripe; Ma, macronuclei; Mi, micronucleus; N, nuclear group; SK, somatic kineties. Scale bars in 1, 3 = 150 μm; in 2, 4 = 100 μm; in 10, 11 = 10 μm; in 12, 13 = 40 μm.

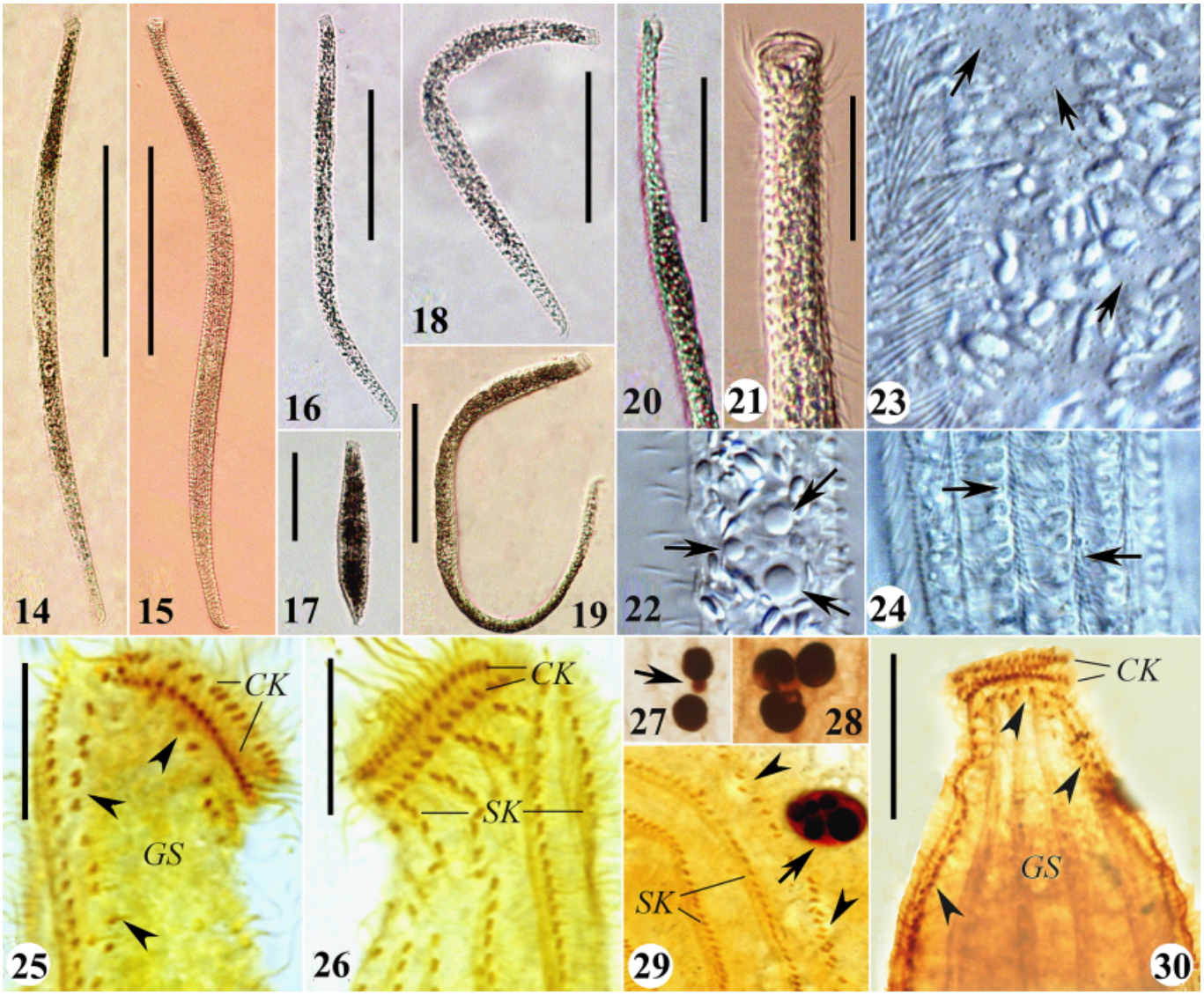

Fig. **14–30.** Photomicrographs of *Apotrachelocerca arenicola* (Kahl 1933) n. g., comb. n. from life (14–24) and after protargol impregnation (25–30). **14–16.** Views of different individuals. **17.** An extremely contracted cell. **18, 19.** Showing flexible body. **20, 21.** Lateral (20) and right (21) view of anterior region to indicate flattened ribbon-like body shape. **22.** Detail of middle region marking nuclear group (arrows). **23.** Distribution of cortical granules in glabrous stripe (arrows). **24.** View of mid-region of right side to show rough surface (arrows). **25, 26.** Left (25) and right (26) side of anterior region, noting glabrous stripe, somatic kineties, bristle kinety (arrowheads), and continuous (uninterrupted) circumoral kineties composed of two rows of dikinetids. **27, 28.** Detail of nuclear group. Arrow in (27) indicates micronucleus. **29.** Lateral view of mid-region marking somatic kineties, bristle kinety (arrowheads), and one nuclear group (arrow). **30.** Infraciliature of left side to indicate glabrous stripe as wide as body width and bordered by bristle kinety (arrowheads). CK, circumoral kineties; GS, glabrous stripe; SK, somatic kineties. Scale bars in 14–16 = 150 μm; in 17–21 = 100 μm; in 25–26 = 10 μm; in 30 = 20 μm.

Usually there are two or three macronuclei and one micronucleus forming a nuclear group located almost in the middle of the cell (Fig. 1, 6, 7, 9, 13, 22, 27, 28). Only one of 31 stained specimens examined was found with one macronucleus but no micronucleus is visible (Fig. 8, 29). The macronuclei are 3–5 μm in diameter and contain many large chromatin aggregates, possibly nucleoli (Fig. 6–8, 29).

Molecular data and the phylogenetic position of the new genus (Fig. 31). The partial SSU rRNA gene sequence of *A. arenicola* is 1,581 bp in length and has been deposited in the GenBank database with accession number JF800908. Phylogenetic trees inferred from the SSU rRNA gene sequences using two methods generate similar topologies; hence only one tree is presented here (Fig. 31). Both ML and BI analyses recover the class Karyorelictea as a fully supported monophyletic group that forms a sister group to the class Heterotrichea. Within the class Karyorelictea, the trachelocercids form a monophyletic clade with moderate supports (0.91 BI, 87% ML), in which *A. arenicola* occupies a basal position.

DISCUSSION

Identification of *Apotrachelocerca arenicola*. This organism was first reported by Kahl (1933) who provided a rather schematic figure (Fig. 2) and a short description: ''Size about 300 μm; body flattened; biotope: sand bottom.'' More information can be

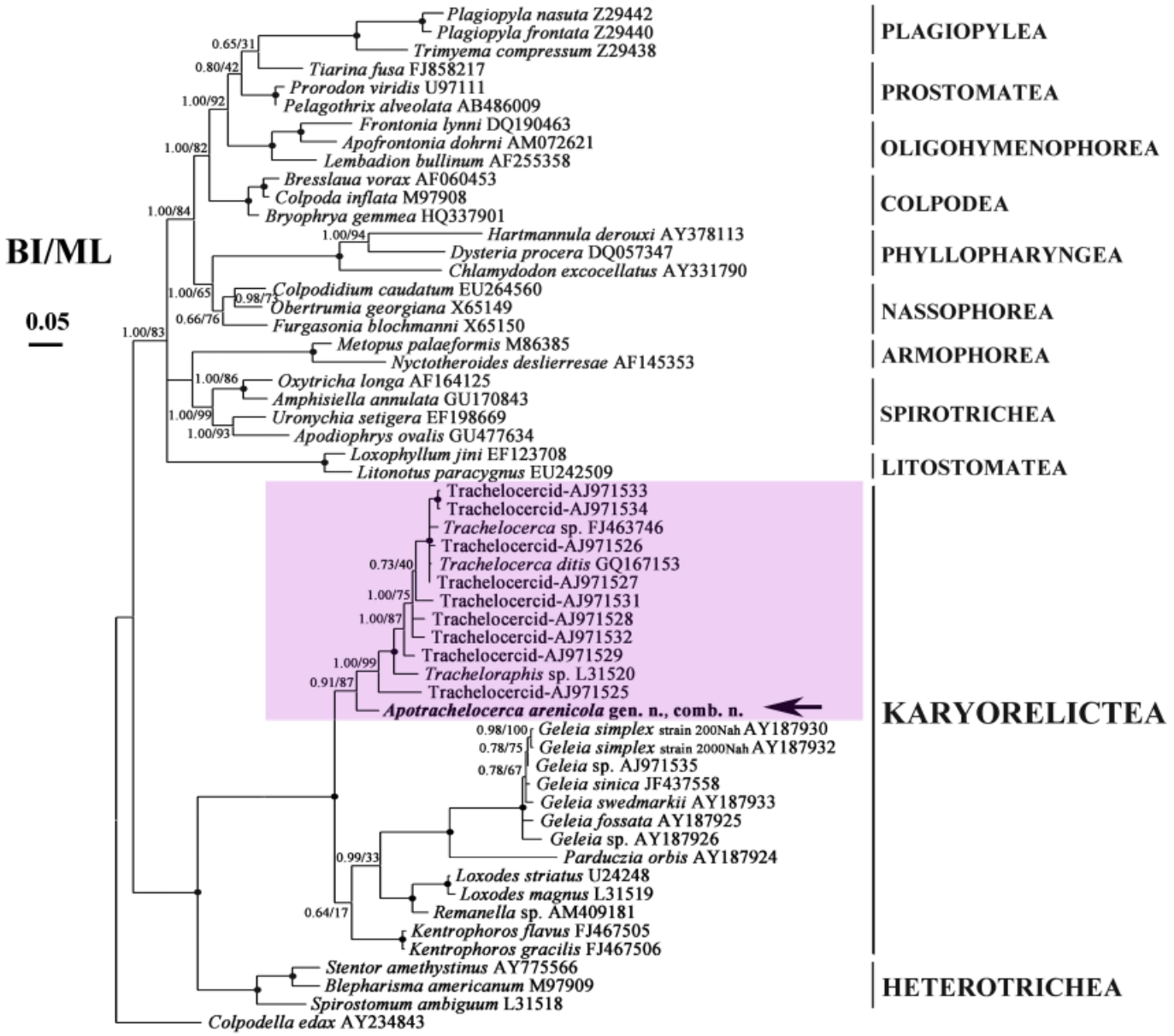

Fig. **31.** Bayesian tree inferred from small subunit rRNA gene sequences showing the position of *Apotrachelocerca arenicola* n. g., comb. n. among the trachelocercid karyorelicteans. Numbers at nodes represent the posterior probability of Bayesian analysis and the bootstrap values of maximum likelihood out of 1,000 replicates. The scale bar corresponds to five substitutions per 100 nucleotide positions. Newly sequenced species in this work are in bold (arrow).

interpreted from his original figure (Fig. 2): broad anterior end; no distinct neck; narrowed and curved tail; and a single nuclear group. Considering all these characters, the Qingdao population corresponds closely to the original form in terms of body shape and size, the general appearance in vivo, and single nuclear group.

Family assignment and comparison. According to Foissner and Al-Rasheid (1999a) and Foissner and Dragesco (1996b), the characters used for generic classification of trachelocercid karyorelictids are the shape and structure of the oral ciliature (Fig. 32–37) and the position of secant system (Fig. 38). Foissner (1996) erected a new monotypic family Prototrachelocercidae within the order Trachelocercida, which is characterized by compound circumoral ciliature comprising more than one row of dikinetids. This new genus *Apotrachelocerca* undoubtedly belongs to this family as its circumoral ciliature is composed of two rows of dikinetids. It differs from *Prototrachelocerca* in that its circumoral kineties are not interrupted (vs. interrupted by brosse kineties in *Prototrachelocerca*).

Another unique or uncommon feature of *A. arenicola* within trachelocercids is that it has no anterior or posterior secant system. However, whether this character is a genus-level one or not remains unknown until more species of this genus are studied in future.

Phylogenetic position of the new genus. The phylogenetic analyses based on SSU rRNA gene sequences revealed that *Apotrachelocerca* fell in the monophyletic trachelocercid clade and was basal to other trachelocercids. Considering that there are no molecular data for *Prototrachelocerca*, *Trachelolophos*,

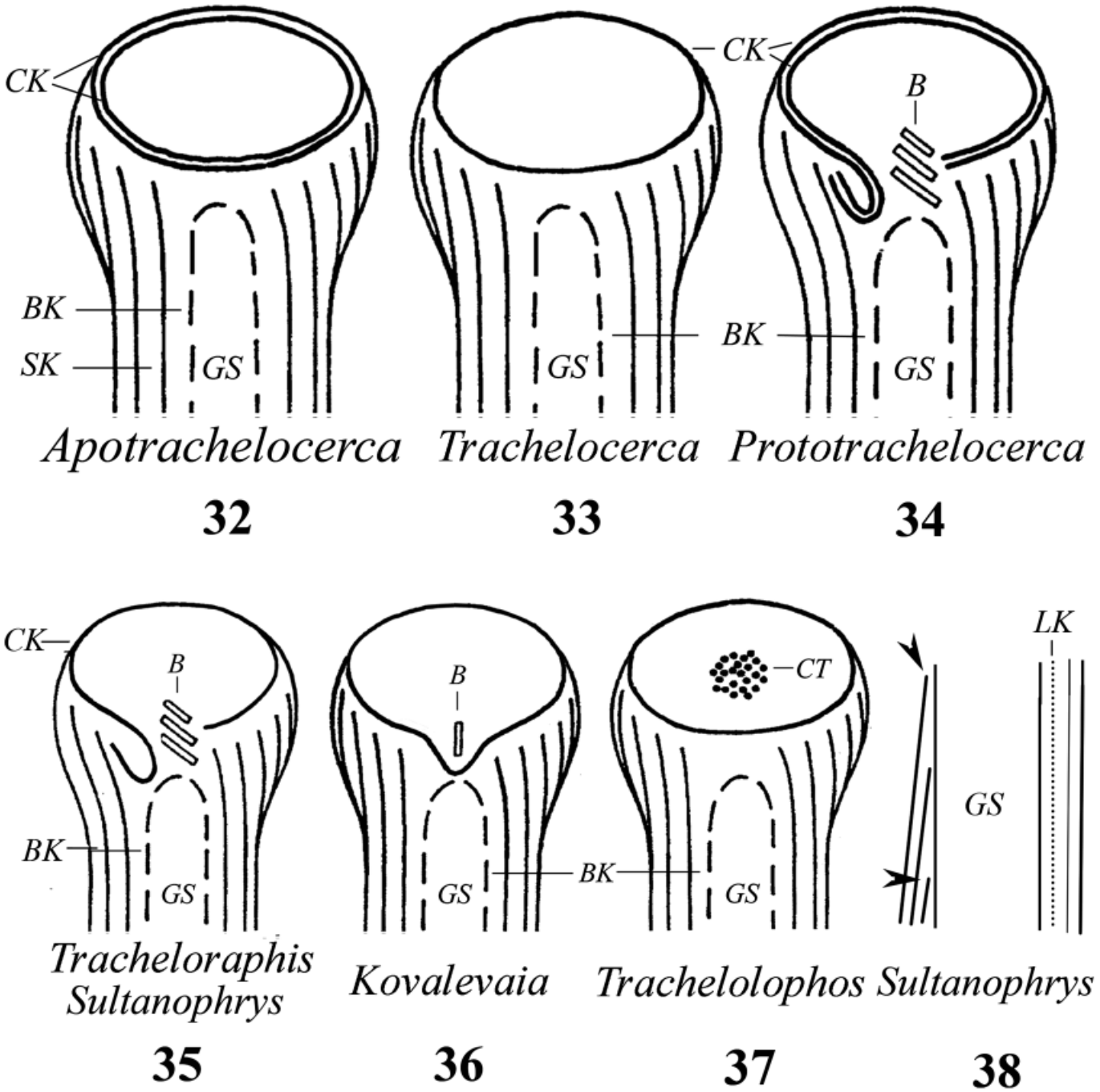

Fig. **32–38.** Features used for generic classification of trachelocercid karyorelictids. **32.** Oral structure of *Apotrachelocerca* n. g. **33.** Oral structure of *Trachelocerca* (from Foissner 1997). **34.** Oral structure of *Prototrachelocerca* (from Foissner 1997). **35.** Oral structure of *Tracheloraphis* (from Foissner 1997) and *Sultanophrys*. **36.** Oral structure of *Kovalevaia* (from Foissner 1997). **37.** Oral structure of *Trachelolophos* (from Foissner 1997). **38.** Secant system (arrowheads) of *Sultanophrys*. B, brosse; BK, bristle kinety; CT, ciliary tuft; CK, circumoral kineties; GS, glabrous stripe; LK, lateral kinety; SK, somatic kineties.

Sultanophrys, and *Kovalevaia*, it is premature to conclude where the prototrachelocercids are placed. More data, especially sequences of additional related taxa and morphogenetic data, are needed to provide an adequate resolution of the taxonomic placement of these genera.

Order Trachelocercida (Jankowski 1978)

Family Prototrachelocercidae (Foissner 1996)

Genus *Apotrachelocerca* n. g.

Diagnosis. Prototrachelocercidae with uninterrupted circumoral kineties composed of two rows of dikinetids. No brosse or ciliary tuft in oral cavity.

Type species. *Apotrachelocerca arenicola* (Kahl 1933) comb. n. (basionym *T. arenicola* Kahl 1933).

Etymology. *Apotrachelocerca* is a composite of the Greek prefix *apo* (derived from) and the genus-group name *Trachelocerca*. Like *Trachelocerca*, feminine gender.

***Apotrachelocerca arenicola* (Kahl 1933) comb. n.**

Syn. *Trachelocerca arenicola* (Kahl 1933)

Improved diagnosis. Extended cells in vivo about 300–600 × 10–20 μm; body flattened ribbon-like, contractile; head indistinctly set off from trunk; usually two or three macronuclei and one micronucleus forming a nuclear group; seven ciliary rows on right side of the cell; left side unciliated, except for bristle kinety; glabrous stripe as wide as trunk; no anterior or posterior secant system at both sides of glabrous stripe; cortical granules minute and colorless.

Neotypification. Because (i) no type material is available and (ii) the original description is simple (only cell size and shape) and some important taxonomic features (e.g. infraciliature) are lacking from Kahl's type population, it is reasonable to designate a neotype. According to Article 75.3 of the International Commission on Zoological Nomenclature (ICZN) (1999), the valid designation of a neotype has to be accompanied by the publication of some particulars:

(i) The taxonomic status of *A. arenicola* is unclear (see discussion for details). In addition, details of the type locality are not known yet.

(ii) For differentiation of *A. arenicola* from related species.

(iii) The neotype (Fig. 12, 13 and its population are described in detail, including some relevant morphogenetic stages accompanied by the SSU rRNA gene sequence. Thus, recognition of the neotype is ensured.

(iv) No type material is available from species originally described by Kahl (1933). Thus, the present species was not objectively defined via a type specimen so far.

(v) There is very strong evidence that the neotype (Fig. 12, 13) is identical with *T. arenicola* Kahl 1933 as originally described by Kahl (1933).

(vi) The original type locality is an unspecified area of the sandy shore of Schilksee Bridge whereas the neotype material is from the Shilaoren sandy beach at Qingdao (China). Although these two places are far away from each other, both sites are marine habitats, and numerous free-living ciliates—especially marine ones, which live in a comparatively homogenous medium—are apparently cosmopolitan, so that this point should not be overinterpreted (Foissner 2002). A detailed description of the new type locality is given in ''Material and Methods.''

(vii) The protargol-impregnated slide containing the neotype specimen is deposited in the Natural History Museum, London, UK. Two other slides containing specimens of the neotype population are deposited in the Ocean University of China (No. XY09100901).

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